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Aldosterone Binding with Corticosteroid Brain Receptors in Rats: Effect of Behavioral Typology and Stress

Yu. A. Akimov, M. V. Onufriev, N. A. Lazareva, and N. V. Gulyaeva

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Differences in ^{3}H-aldosterone binding with hippocampus cytosol receptors were found to be dependent on the behavioral type of male Wistar rats in the "emotional resonance" test. These differences were not observed in the cytosol analysis of the remaining part of the brain. Control rats and rats subjected to short-term stress by painful electrical stimulation showed a long-term drop of ^{3}H-aldosterone binding with hippocampus cytosol in active as compared to passive animals preferring a closed space.

Key Words: *corticosteroid receptors; aldosterone; brain; hippocampus; stress; individual behavior*

In recent years a great deal of attention has been paid to the role of brain corticosteroids (including aldosterone, AS) and corticosteroid receptors (CR) in various functions of the central nervous system. Steroids are able to directly interact with the neuron membrane, but most of the effects of glucocorticoids and mineralocorticoids are determined by their binding with intracellular receptors and subsequent alterations in the expression of certain genes [7]. The highest CR density in the brain is specific for the limbic system and above all the hippocampus. Mineralocorticoid receptors respond to the same signal as glucocorticoid receptors (corticosterone or cortisol). Mineralocorticoid receptors of the limbic system are believed to interact predominantly with corticosterone even if the blood level of the latter is very low [7]. CR of the limbic system and other brain areas maintain the ionic balance, reactions to neurotransmitters, and neuron excitability, as well as participate in the stress response, information processing, emotional reactions, and the execution of behavioral strategies [7,9].

These findings indicated that the brain CR probably play a role in individual behavior and prompted an investigation of AS-CR binding in the brain of rats with different characteristics of behavior. The "emotional resonance" phenomenon (ER) is a useful tool for behavioral typology in rats [3]. Since it was shown earlier that neurochemical differences among animals divided in the ER test were more pronounced after short-term stress [2], it seemed advisable to study both the basal and post-stress characteristics of the brain CR.

Laboratory of Functional Biochemistry of the Nervous System, Institute of Higher Nervous Activity and Neurophysiology, the Russian Academy of Sciences, Moscow (Presented by I. P. Ashmarin, Member of the Russian Academy of Medical Sciences)

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MATERIALS AND METHODS

One hundred fifty male Wistar rats weighing 120-150 g were subjected to a single ER test [2]. Rats placed in an open (illuminated) space had the choice either to remain there or to move to the closed (dark) space. The moment a rat found itself in the closed space it let out a cry, being subjected to unavoidable painful electrical stimulation. The victim's cry ceased on emergence into the open space. Testing continued for 300 sec; the number of visits and time spent in each field were recorded. Two groups of animals comprising 36 rats each were used for further study, the first group consisting of passive rats preferring the closed space (one visit, time in the open space 4.6±0.2 sec) and the second group of active rats (7.5±0.2 and 121.3±9.8, respectively). Half of the rats were subjected to unavoidable pain stress (10 shocks of 6-sec duration, current strength 10 mA, total stress time 15 min) [10]. The animals underwent a bilateral adrenalectomy under ether narcosis three days prior to decapitation.

Two weeks after stress the rats were decapitated and the hippocampus and brain without the hippocampus and cerebellum were promptly excised (the left and right halves separately) and homogenized in six (hippocampus) and five (brain) volumes of medium containing 10 mM Tris-HCl, 0.25 mM sucrose, 2 mM dithiothreitol, and 3 mM $MgCl_2$, pH 7.6, using a Potter homogenizer. Homogenates were centrifuged at 105,000 g for 90 min. Supernatant samples were incubated with ^{3}H-AS for 20 h (final concentration 15 nM) in the presence or absence of a 1000-fold excess of AS. The samples were treated with a mixture of Norit-A 4% carbon and 0.5% dextran (molecular weight 80,000 D) in 1 M sucrose for 5 min to remove unbound ^{3}H-AS. Samples freed of solid particles were placed in scintillation flasks. Radioactivity was measured on an SL-4000 scintillation counter (Intertechnique). All procedures were performed at 2-4°C. Protein was determined by the method of Bradford [6]. Reagents were provided by Serva (AS, Norit-A, $MgCl_2$), Koch-Light Lab. (dithiothreitol, Tris), and Amersham (^{3}H-AS). STATGRAPHICS statistical analysis was performed using a standard software package.

RESULTS

Specific binding of ^{3}H-AS was higher in the brain and hippocampus of passive rats (preferring the closed space in the ER test), but both groups showed a stress-related decrease in ^{3}H-AS binding

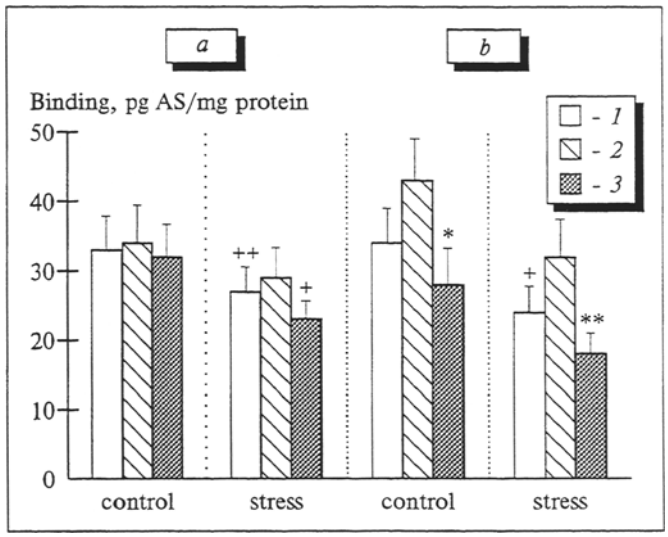

Fig. 1. ^{3}H−AS binding with cytosol receptors of the brain (without the hippocampus and cerebellum, *a*) and hippocampus (*b*). The whole (*0*) group of animals (all controls and animals subjected to stress) and the first (*1*) and second (*2*) groups. $^*p<0.05$, $^{**}p<0.02$ as compared with the first group; $^+p<0.05$, $^{++}p<0.03$ as compared with the control using Student's *t* test.

(Fig. 1). Statistically significant differences in ^{3}H-AS binding in the control group and stressed animals were observed only in the hippocampus of animals with different types of behavior. Low protein levels in the hippocampus cytosol are the reason for the similar parameters of ^{3}H-AS binding in the hippocampus and brain; the same protein levels afford a reliably higher binding with the hippocampus receptors as compared with receptors of other parts of the brain [7].

Analysis of variance (ANOVA) demonstrated ^{3}H-AS binding in the hippocampus to be dependent on stress (F=4.96, p<0.03) and type of behavior (F=9.72, p<0.004), although the interplay of these factors was statistically insignificant (F=0.44, p>0.7). ^{3}H-AS binding in the brain was found to depend only on stress (F=5.27, p<0.03) and not on type of behavior (F=1.08, p>0.2).

According to Sapolsky [9], under conditions similar to those of the present study the ^{3}H-AS level is higher in mineralocorticoid receptors and lower in glucocorticoid receptors colocalized in the majority of hippocampal neurons [11] and glial cells [5]. It is currently thought that the hippocampus plays a specific role as a "crossing point" between consciousness and emotions (stress being an example), which is why the hippocampus has proved to be one of the most important objects for the study of stress-related events [8]. We did indeed succeed in demonstrating a specific dependence of ^{3}H-AS binding with hippocampus cytosol receptors on behavioral type. The effect of stress was revealed both in the

hippocampus and in the rest of the brain. CR are known to determine the hyperpolarization of CA1 hippocampal neurons that is connected with the 5-HT1A serotonin receptors [4]. Differences in ^{3}H-AS binding in the hippocampus may be caused by different amounts of *de novo* synthesized serotonin and other monoamines in the rat brain which are characterized by similar individual and typological behavioral features in the ER test [1].

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